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A REPORT ON DISEASE DIAGNOSIS OF WHITE SPOT SYNDROME (WSSV) SURVEILLANCE PROGRAMME CARRIED IN NELLORE DISTRICT- ANDHRA PRADESH -2017

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ABSTRACT

India is endowed with a wide diversity of water resources, which can provide scope for highly developed fisheries sector. The sector provides nutrition and livelihood security for a large number of people and also source of foreign exchange. With rapid shift from capture to culture fisheries, the sector needs systematic intervention on aquatic health problems. White Spot Syndrome (WSSV) is prevalent in L. vannamei but diagnosis can help farmers avoid and reduce the spread of WSSV to other ponds. The early harvest just by seeing the white spots on carapace can be avoided by implementing these diagnostic techniques. Significant proportion of brackish water farms are adopting poly-culture Biological Samples Screened and Culture. A sample size of 51 were screened for nine pathogens and diseases were detected and revealed are three diseases pathogen namely, IHHNV, WSSV and EHP. The appearance of white spots on shrimp is predominant, causing panic to farmers and often resort to emergency harvest and to save whatever they can. The present work helps in differential diagnosis of white spots at the laboratory level and confirmatory diagnosis by using rapid test kits like rapid dot or Shrimple. On the other hand, it is necessary that the farmers should maintain strict biosecurity measures to prevent the spread of the diseases to the other species.

INTRODUCTION:

India is endowed with a wide diversity of water resources, which can provide scope for highly developed fisheries sector. It has a long coastline of 8118 KM and a huge 1.9 lakh KM area of inland water resources with a water spread area of 7.4 lakh hectares. The sector provides nutrition and livelihood security for a large number of people and also source of foreign exchange.

As other animals, aquatic animals are also prone to diseases and need intervention and surveillance. This is needed for improving trade with other countries as also for protecting our resources. With rapid shift from capture to culture fisheries, the sector needs systematic intervention on aquatic health problems. As more and more demand for fish is growing at a rapid rate. To meet this growing demand, production system has to be move to semi intensive and intensive from traditional system. This move will result in frequent diseases. To meet this situation, development of health surveillance system becomes important.

Among the fishery products traded internationally Shrimp is counted as one of largest seafood commodity accounts to 17%. Penaeus monodon and Litpaneous vannamei are major species dominated in the culture area, it contributing nearly 75% of production in aquaculture sector and these two are also represent under invertebrate as a substantial food animal (FAO, 2009, Televisory 2020).

Shrimp is major product in marine exports. It is culture in an extent of about 21 thousand hectares and its production is estimated to 3.75 lakh MT during 2019-20 . Andhra Pradesh is the major contributor of shrimp in the country with a share of 58.7 per cent. But its share in area is only 31.2 per cent. Thus, the state achieved high productivity in shrimp production. Thus, Andhra Pradesh stands at the top in both production and productivity

Andhra Pradesh is known for shrimp production and the state contributes 60 per cent of all India shrimp production.

White Spot Syndrome (WSSV) Though very few culture farms are permitted for culture of L. vannamei in India with strict biosecurity conditions and supplying the healthy seed from specific pathogen brood stock, still there are disease out breaks such as White Spot Syndrome (WSSV) in most of the cultured farms. Hence an attempt has been made in the present study to confirm whether really the WSSV is prevalent in L.vannamei or not and to identify a suitable and easy method of diagnosis for the farmers using the available techniques like, molecular diagnostics Polymerase Chain Reaction (PCR) and isothermal PCR), histological (Histopathology using stained sections of hematoxylin and eosin and rapid gill staining), immunodiagnostics (Rapid dot kit and Shrimple kit), microscopic and clinical observations (Differential Morphology of white spots and the variations in the morphology of white spot between juveniles and sub adults). The early and accurate diagnosis of these diseases may help farmers to avoid and spread of WSSV to other ponds, can reduce the mass mortality. Also, the early harvest without WSSV infection just by seeing the white spots on carapace can be avoided by implementing these diagnostic techniques (M.A.Badhul Haq et al., 2016)

MATERIAL AND METHOD:

Sample Size:

A total sample of 51 samples from brackish water covering Nellore districts were collected from various farmers of Andhra Pradesh.

Sample collection:

The survey was covered from different sources namely, hatcheries, nurseries, rearing ponds and grow-out ponds. The number of grow-out ponds were more as there are not separated from nursery and rearing ponds. Seed is the major source for impact on disease outbreak. It is significant to note that a significant proportion of brackish water farms are also adopting poly-culture.

Biological Samples Screened and Pathogens Detected:

It is found that three diseases namely, IHHNV, WSSV and EHP were dominant disease. IHHNV is the most predominant disease found in 61.4 per cent of the cases, followed by WSSV found in 38.6 per cent and new disease EHP in 20.3 percent of the cases. The results indicate that WSSV is dominant during this period.

Method:

PCR methods for WSSV and IHHNV was followed the procedures of IQ 2000 KIT method. Isothermal PCR for WSSV was performer as per the procedure of IQ screen kit manufactured by Framing Intelligence Tech. Corp. Taiwan.

Physical Observation:

The prevalence of WSSV infection in exotic species, L. vannamei in spite of its Specific Pathogen Free(SPF) status of brood stock and high health post larvae. Unlike P. monodon, in L. vannamei white spots are not visible externally due to the white colour of the shrimp (Figs. 1 and 2). In addition to that white spots are present in almost all the cases of mortality might be due to the prevalence of other diseases like vibriosis (Fig. 3), in healthy shrimps (Fig. 4), moulted shells (Fig. 5) and thus making difficult to take decision of harvesting (Early harvest may prevent the spread of the virus to the other ponds of that farm and among other farms and can also reduce the loss). In several cases it was observed that the harvesting has been performed without WSSV outbreak by just observing the white spots on the carapace and even during the mortality because of other problems.

In addition to above, the signs and symptoms like anorexia, redness of the body (Fig. 1), antennae cut (Fig. 6), surfacing of shrimp, cannibalism (Fig. 7), oedema in the cephalic region (Fig. 8), pre and post moult death were also observed. However, these symptoms are not common in all the cases of WSSV disease, similar signs were also reported in other diseases (Lightner et al., 2006) Table 1 , making it difficult to take a decision. Hence, an attempt has been made with different available confirmative diagnostic procedures to evaluate and choose the best techniques as given below.

a. Polymerase chain reaction
b. Isothermal PCR
c. Histology
d. Rapid gill staining technique
e. Rapid dot kit
f. Shrimple kit

g. Morphology of white spots developed

a. Polymerase Chain Reaction:
The shrimps (10 samples) suspected to be infected with WSSV having external symptoms of white spots and mortality were analyzed, and found that only 5 samples are positive by PCR and I screen isothermal PCR system (Fig.13). This clearly evidences that all the white spots observed in cultured shrimps are not caused by WSSV. Even the ponds, which are positive by PCR and isothermal PCR, survived for more than one month without any visible symptoms of morbidity and mortality by implementing the effective management practices. Thus the PCR and Isothermal PCR techniques are very much useful for early diagnosis of the disease and to prevent the spread of the virus to other ponds and other culture areas. In one particular case of our study, the post larvae were observed positive for WSS within 5 days of stocking and survived for 35 days without any signs and symptoms of WSSV, this could be possible by the efficient pond management practices. This study conforms the PCR and isothermal PCR can be used to detect and differentiate mortality caused by WSSV.

b. Histography of gill:
The same samples used for PCR were fixed in Davidson fixative and processed for histopathology using hematoxilin and eosin stains. 5 samples show the characteristic intranuclear cowdry type inclusion bodies as shown in fig.9 confirming WSSV infection. This study is time consuming procedure, requires minimum 5 days time for getting final result and sometimes even mortality may start before getting the final result, as the incubation time is less than 5 days for some virulent strains of WSSV (OIE, 2009). The histopathological symptoms of early stages WSSV resembles the histopathology of IHHNV in formation of eosinophilic intranuclear cowdry a type inclusion bodies (OIE, 2009)

c. Rapid Gill staining technique:
The gills from the moribund shrimps were collected and processed for rapid gill staining out of ten samples only three were showed hypertrophied nuclei and it is also a characteristic feature of WSSV infection (Fig. 10). This technique is cost effective but it requires more laboratory support.

d. Rapid Dot kit:
Rapid dot kit is relatively high sensitive than shrimple kit and it is able to detect the infection at least 15 days in advance before the onset of mortality. Based on the intensity of the color development viral load can be assessed and it is relatively easy and economical, at a time four samples can be analyzed at pond side with in 10 minutes.(Fig 11)

e. Shrimple kit:
Shrimple kit is a less sensitive technique than PCR, isothermal PCR and rapid dot kit. It can detect the infection three to four days in advance and very good tool for making a harvest decision. The procedure is relatively easy and kit is little bit expensive than the rapid dot kit. (Fig 12) illustrates presence of red band at point T confirmation WSSV infection. The advantages and disadvantages of the different kits used in the present experimental study were depicted in the table-2.

f. Chromatophore identification:
The pleopods of the infeted shrimps with WSSV were observed under the microscope for observing the chromatophores, they were first turned to yellow (Fig. 14), and the colour gradually turned to red (Fig. 49), indicating the prealence of viral stress in the cultured shrimps and this pattern of change in colour can be taken as an indicator.

g. Microscopic identification of White spots:
During the culture practice, in most cases the farmers rely on white spots exhibiting on the exoskeleton of shrimp as the specific diagnosis symptom for this most dreaded WSD and resort to an emergency harvest without knowing the actual cause. Hence, it is aimed to study the morphology of white spots caused by different reasons to compare the etiologies. The samples with visible white spots on the carapace were observed under the microscope, the different morphological characteristics as follows. The carapace of the L.vannamei those are positive by shrimple kit for WSSV was observed under microscope. The morphology of white spot caused by WSSV in L. vannamei is with a hole in the center and radiating lines (Figs. 17 and 18) is different from the white spot morphology of P.monodon with dense melanized dots (Fig. 19).Only one case of shrimp mortality suspected with Vibrio infection was reported during the research work. The white spots that were present on carapace were observed under microscope for morphological studies. The morphology caused by WSSV was different from the morphology of white spot caused by vibrio spp. (Fig. 20). This morphology of the white spot caused by Vibrio resembles the one reported by Cyrille Goarant et al. (2000) in L. stylirostris caused by bacteria. The carapace of P. vannamei juveniles with confirmed WSSV infection was observed for morphology. The morphology of white spot caused by WSSV in juveniles (Fig. 21) was different from sub adults and lacking central hole but the radiating lines were present.

Exuvia of the shrimp from the healthy ponds were collected, stored in the same pond water and brought to the laboratory. The morphology of white spot present in almost all the moulted shell of healthy ponds without causing mortality was different with one or two homocentric rings and darkened center with floral structure (Figs. 16 and 22) and resembles the morphology of described by Wang et al. (2000) in P. monodon caused by the Bacillusspp. It is a well-accepted fact that WSSV is endemic to India. During the present study, it has been observed that due to lack of biosecurity at the farm level, like crab fencing, bird fencing, pumping water directly from creaks without treatment for disinfection and filtration to prevent the entry of carriers are the main reasons suspected for the present outbreak of WSSV.

In a particular case during the present investigation, it was observed that *P. monodon shrimp entered the L. vannamei pond prior to stocking due to the* lack of filtration and for not treating the water in reservoir with disinfectants for eradicating the carriers for white spot virus. White spot disease first affected the *P. monodon and later spread to L. vannamei* (Fig. 23). From the fig. 23 it was clearly evident that the size of P. monodon is been bigger than the L. vannamei,so it indicates that P. monodon entered the pond before stocking the target species and first mortality has been started with P. monodon. The white spots are clearly visible on the peeled carapace of the P. monodon, whose morphology is almost similar to the morphology of white spot (Wang et al., 2000).

Table 1: signs and symptoms observed in white spot syndrome

Sign	No of cases	Other Problem with same sign
Redness	15/20 cases	Low do, vibrio, stress
Antennae Cut	10/20 cases	Vibriosis
White Spots on the Cuticle	20/20 cases	Bacterial spot
Surfacing	10/20 cases	Low do
Cannibalism	18/20 cases	All diseases
Oedema in cephalic region	2/20 cases	Low do
Pre and post molt death	10/20 cases	Ammonia toxicity, DO deficiency
Anorexia	15/20 cases	All cases of stress

Table 2: Comparison of different diagnostic procedures for WSSV

Diagnostic procedure	Advantages	Disadvantages
PCR	Highly sensitive	Requires sophisticated lab
Isothermal PCR	Highly sensitive	Requires I screen oven
Histology	Confirmatory	Requites lab/time consuming
Rapid gill Staining	Confirmatory	Requites lab support
Rapid dot	Good for harvesting decision	Storage at 4ºC and
Shrimple Kit	Good for harvesting decision	Relatively expensive

RESULT AND DISCUSSIONS:
Observed that the following factors for the occurrence of disease outbreak:

(i) Poor quality of seed

(ii) Poor water quality and management

(iii) Fluctuations in the environmental conditions like temperature., release of toxic gases etc.

(iv) Poor pond management between crops

(v) Inadequate bio-security measures and high stocking densities

(vi) Horizontal transmission of pathogens through carriers

CONCLUSION:
It is desirable to establish relationships between soil, water, seed and feed quality parameters and chances of disease outbreak.

1. A large number of samples were screened for nine pathogens and diseases were detected and revealed are three diseases pathogen namely, IHHNV, WSSV and EHP. It may be concluded that WSSV are highly prevalent diseases during the phase.

2. It is suggested that the state departments should recognize some of the private diagnostic laboratories with adequate facilities and bringing them into the fold of regular disease surveillance program for monitoring disease outbreaks in the state of Andhra Pradesh.

3. Capacity building of all staff involved in disease surveillance programme and periodical feedback from the farming community, proper data management, reporting mechanism and follow up actions are important to strengthen the disease management.

4. The high growth in the recent period is accompanied by intensification and adoption new seed, feed and production technologies. With high demand in the export market, production is also shifting towards shrimp and other finfish from carp. These changes have also contributed to frequent occurrence of diseases. Export of any livestock product has to satisfy OIE and WTO obligations. As per these standards, every country has to submit a quarterly report to OIE about occurrence of diseases.

5. It is envisaged that outbreak of white spot disease in exotic species L.vannamei. The appearance of white spots on shrimp causing panic to farmers and often resort to emergency harvest and to save whatever they can. However, there were some incidences, farmers avoided the emergency harvest and got successful crop. In general, the White spots could be due to the infection of WSSV, Vibrio spp. and Bacillus species. The present work helps in differential diagnosis of white spots at the laboratory level and confirmatory diagnosis by using rapid test kits like rapid dot or Shrimple. On the other hand, it is necessary that the farmers should maintain strict biosecurity measures to prevent the spread of the diseases to the other species

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Figure 1: *L. vannamei* infected with WSSV and white spot are not visible externally. The Red colour of the shrimp could be possibly due to the expansion of chromatophore

Figure 2: White spot present on the carapace of *L. vannamei* infected with WSSV.

Figure 3: White spot present on the carapace of *L. vannamei* infected with vibriosis and WSSV negative.

Figure 4: White spot present on the carapace of healthy *L. vannamei*

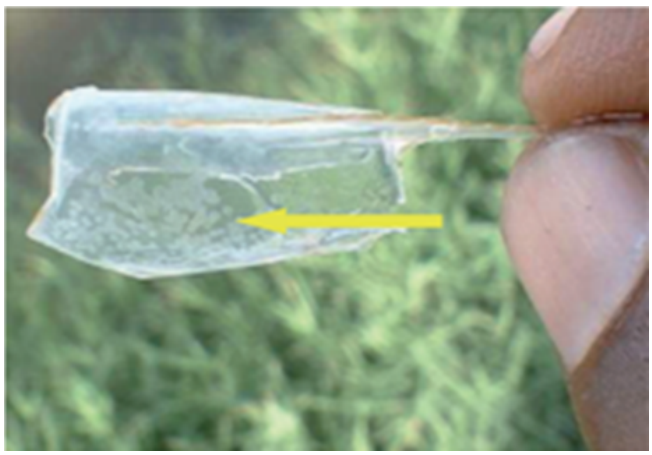

Figure 5: White spot present on the moulted carapace of *L. vannamei* from a healthy pond

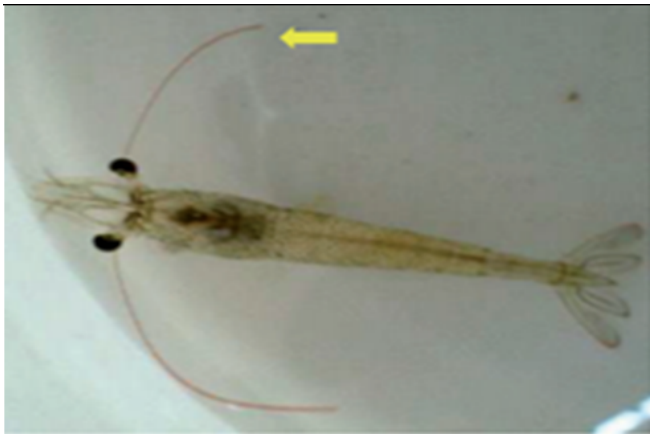

Figure 6: *L. vannamei* juvenile with cut antennae

Figure 7: Cannibalized shrimp *L. vannamei* in check tray of WSSV infected pond

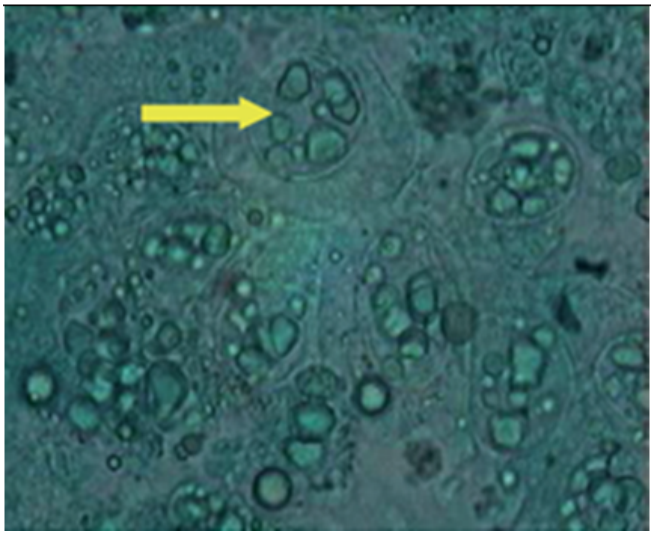

Figure 8: Oedema in cephalic region of *L. vannamei* Infected with WSSV

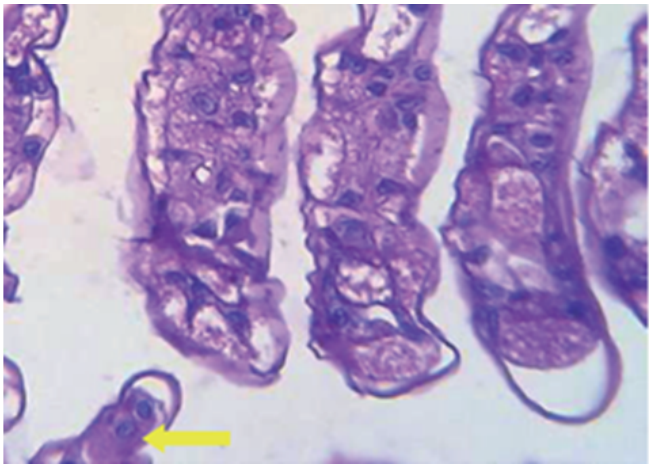

Figure 9: Histopathology of gill showing intranuclear inclusions. Characteristic of WSSV infection

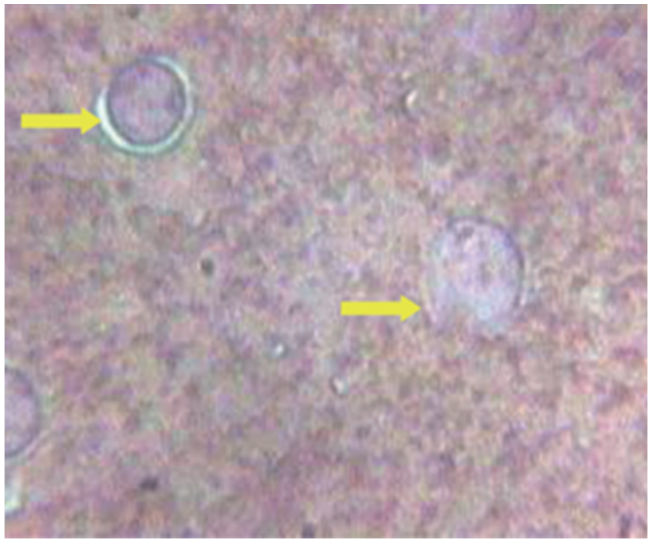

Figure 10: Rapid gill staining of epithelial tissue of Litopenaeus vannamei showing hypertrophied Nuclei

Figure 11: Rapid dot kit showing negative result for WSSV in the Litopenaeus vannamei sample with white spot with morphology of bacteria white spot.

Figure 12 : Shrimple kit showing WSSV positive result.

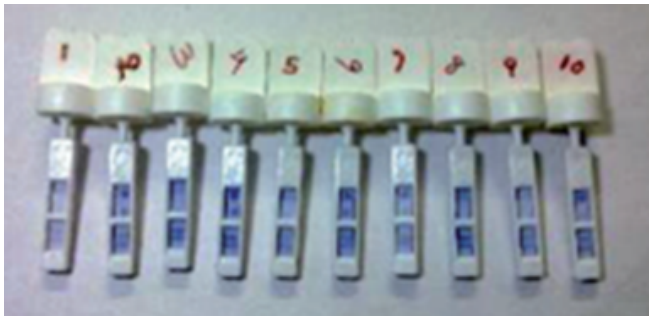

Figure 13: Result of Iscreen isothermal PCR system showing 5 positive and 5 negative samples for wssv in shrimps with white spots

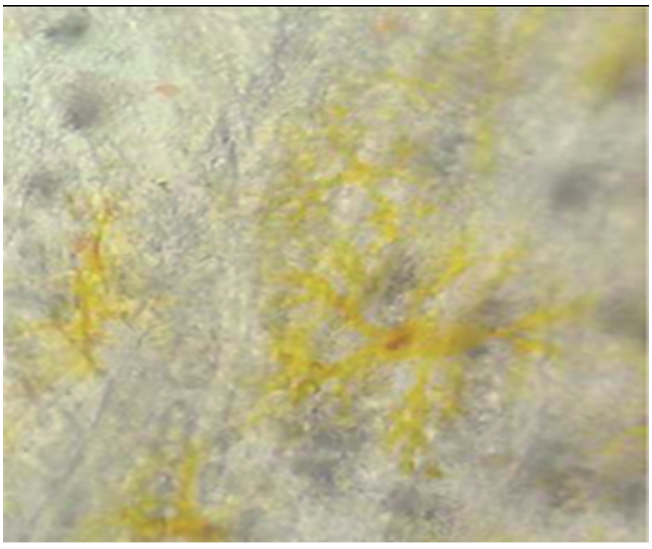

Figure 14: Yellow colour of the chromatophore in the early stage of wssv infection

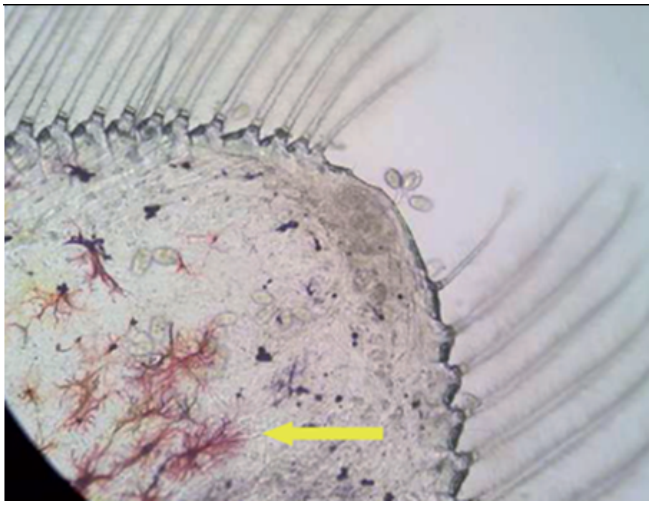

Figure 15: Red color of the chromatophore in advanced stage of the WSSV infestion. Protozoan fouling is also visible

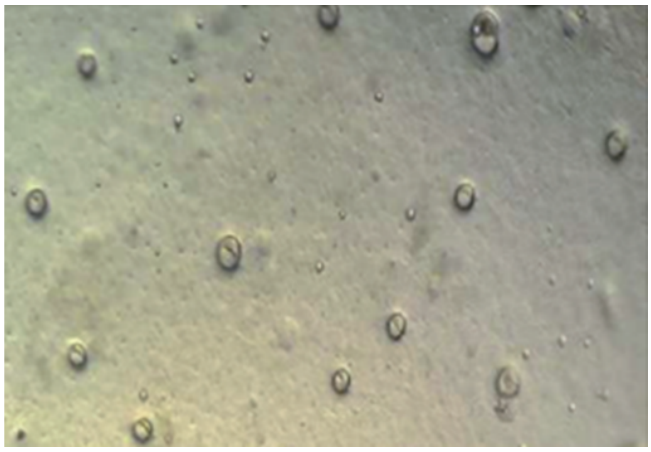

Figure 16: wet mount of normal shell of Litopenaeus vannamei

Figure 17: Early stage of white spot development

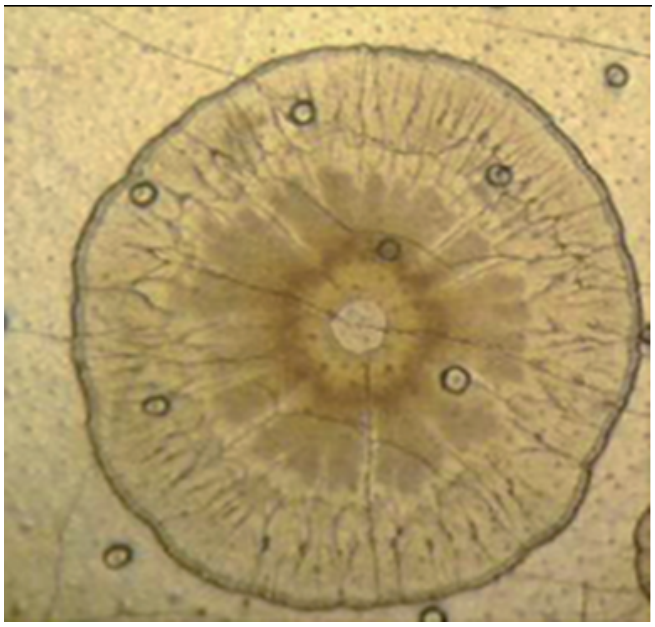

Figure 18: Morphology of white spot in sub adult *L. vannamei*

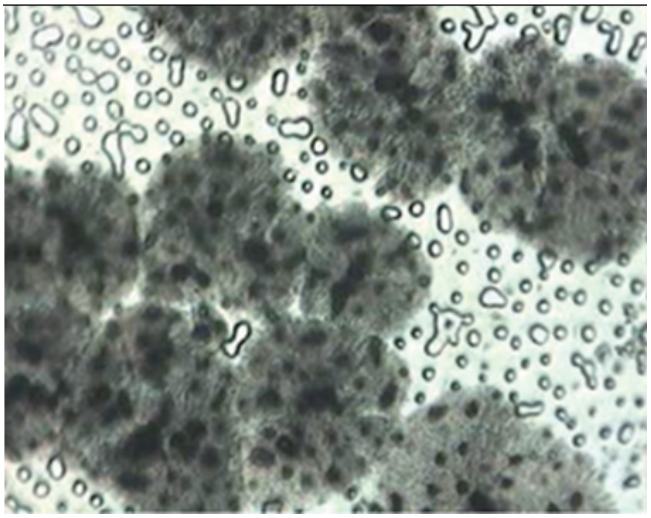

Figure 19: Morphology white spot on Penaeus monodon with WSSV infection

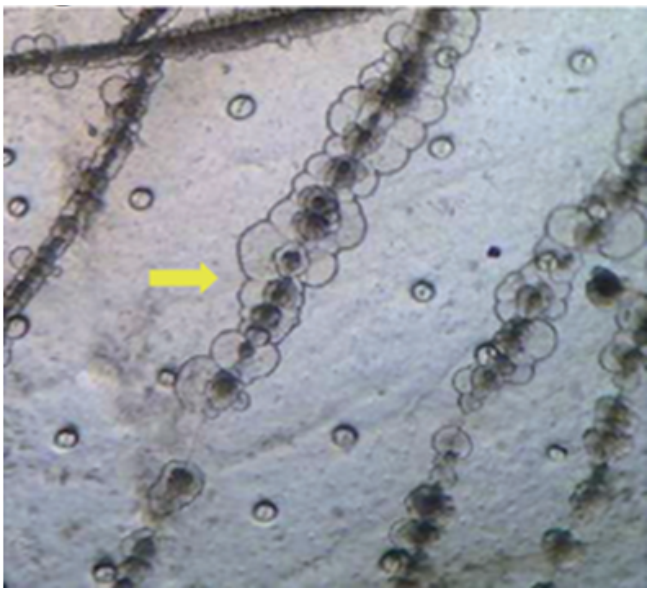

Figure 20: The morphology of white spot caused by Vibrio species in *L. vannamei* (10x)

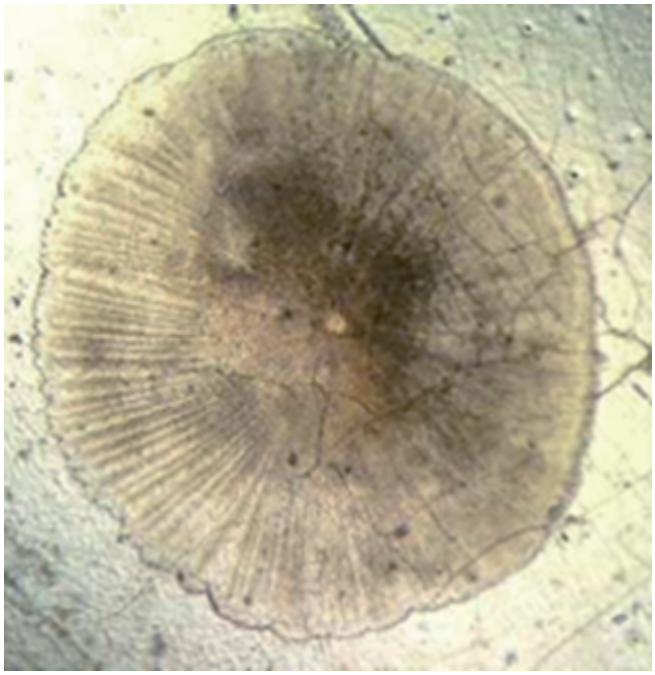

Figure 21: Morphology of white spot in juveniles of *Penaeus monodon*.